REMARKS

Claims 245-255, 258 and 262-269 are pending in the above-referenced application. As will be discussed in further detail below, claims 245, 249, 255, 262, and 268-269 have been amended to more distinctly recite the subject matter which Applicants regard as their invention. As will be discussed in further detail below, these claim amendments are supported by the specification. Claims 256-257 and 259-261 and 263-264 have been canceled. Applicants reserve the right to pursue subsequent continuation and/or divisional applications on subject matter encompassed by the canceled claims.

1. Restriction Requirement

Applicants note the Restriction Requirement issued with respect to claims 268-269. Claims 268-269 have been withdrawn. However, as noted in the Office Action, these claims are subject to rejoinder upon indication of allowable subject matter with respect to the product claims.

2. Sequence Compliance-Drawings

It is noted that the application fails to comply with the requirements of 37 CFR 1.821 through 1.825 since there are sequences in the drawings that do not contain a SEQ ID NO. In response, Applicants note that an amendment was submitted on February 15, 2005. This amendment contained an amendment of the "Brief Description of Figures" where sequences set forth in the figures are identified with SEQ ID NOS. A copy is attached hereto as Exhibit 1 for the Examiner's reference. Thus, compliance has been achieved.

3. The Rejections Under 35 USC 112, First Paragraph (Written Description)

Two rejections were made and are set forth below.

3.1 The Rejection of Claims 245-254, 263 and 264

Claims 245-254, 263 and 264 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. It is

asserted that there is inadequate support for the term "non-eukaryotic polymerase" in claim 245.

Applicants respectfully traverse the rejection. However, in order to advance prosecution, claim 245 has been amended to be directed to a polymerase. Claims 246-254 depend from claim 245. Thus, arguments made with respect to claim 245 apply to these claims as well. Applicants note that claims 263-264 has been canceled.

In view of the above amendments and arguments, Applicants assert that the rejection of claims 245-254, 263 and 264 under 35 USC 112, first paragraph have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

3.2 **The Rejection of Claims 245-255, 258 and 262-267**

Claims 245-255, 258 and 262-267 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Office Action specifically states:

> Applicant asserts that the specification actually does provide guidance as to properties of the intron that would lead to inactivation such as a) having the presence of the intron through the coding sequence out of frame and/or b) using an intron sequence that has at least one stop codon in frame with the target gene sequence or preferably like the SV40 intron, having a stop codon in all three reading frames. Applicant further asserts that the use of this system would be a general strategy that should work for most proteins regardless of what particular function the protein normally carried out. However, it is important to note that the instant claims are not limited to the embodiments addressed by applicant above. The specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any incompatible cell because the specification provides only minimal prophetic description and no exemplification, of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or cells compatible or incompatible for whom known structures exist that could be utilized having the claimed function. As stated above, the specification provides only minimal prophetic description and no exemplification, of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or cells

compatible or incompatible for whom known structures exist that could be utilized having the claimed function. The specification provides for the use of T3, T7 or SP6 polymerases, and also for the use of certain "consensus splice donor and acceptor sites for inserting introns. Applicants prophetically suggest that intron "insertion at any of these sites in a gene coding region should not affect subsequent removal of the processing element in a compatible cell." (page 84 of the instant specification).

However, there is significant unpredictability in such intron removal, since such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery. A review article by Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166). Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the mRNA (or ribozyme) after splicing, applicants claimed nucleic acid constructs, following splicing, would likely therefore contain elements of these exon recognition sites. Such unpredictabitity indicates that the genus of nucleic acid constructs comprising any intron in any polymerase (or any bacteriophage polymerase), or any toxic gene, and that are active or inactive depending on whether they are found in cells that are compatible or incompatible is very large. The fact that the specification discloses only prophetic examples and a few species of polymerases and donor/acceptor splice sites is not considered to constitute a sufficient representative sample of the genus of such constructs.

Although applicant asserts that the examples that Balvay bring up are more in the nature of exceptions to the general rule and that there are normal rules of how and where splicing would occur and are predictable, these are simply assertions that are not supported by the instant specification or the art. The teachings of Balvay et al. support the unpredictability of the splicing mechanism, rather than the presence of "normal rules" that are referred to by applicant. Applicant concludes that Balvay et al. are only saying that sometimes it is not where it would normally be expected to

take place. This statement is considered to support the unpredictability of such a mechanism.

Applicants respectfully traverse the rejection. Applicants note that claim 245 as amended recites that the polymerase is incapable of being expressed in a prokaryotic cell, due to the presence of said intron; claims 255 and 262, as amended, recites that the gene product is incapable of being expressed in a prokaryotic cell due to the presence of said intron. The claims, as amended recite that the presence of the intron is responsible for lack of expression in the prokaryotic cell. Therefore, contra to assertions made in the Office Action, one would not use any intron. The intron used would have to be responsible for lack of expression of the gene product (in the case of claim 245, a polymerase) in a prokaryotic cell. Applicants assert that one of skill in the art would know which features are necessary in the intron in order to possess this function.

Although, Applicants concede a lack of adequate written description issue arises if "the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process"[1], this is not the situation here with respect to the "non-native intron" used in the construct of the present invention. For instance, the simple expedient of determining that the number of nucleotides in the intron would generate a frame shift in the coding is an obvious approach which also indicates that 1/3 of introns chosen at random would have this feature. The presence of stop codons is another easily ascertainable approach since it is well known that one of the notable features of introns are the presence of stop codons. In point of fact, this was one of the features that was used in the early identification of introns as opposed to coding sequences. Thus, it would be clearly evident to one skilled in the art what characteristics in an intron would be appropriate to use in the constructs of the invention .

The Office Action has also questioned whether "any polymerase" would be applicable. It should be noted that the methods used to block expression are not related to the ultimate function of a protein. As such, the presence of frameshifts and/or stop codons in the intron should disrupt the functionality of any protein target and not

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[1] MPEP 2163 I.A.

just for any polymerase. The only requirement would be knowledge of the sequence of the protein or polymerase so an appropriate site could be chosen. Methods that can be applied for disrupting a gene by introduction of an intron are known in the art. Additionally, the present invention has presented a novel method that would maximize the chances of retaining functionality of the polymerase after splicing out of the non-native intron sequences. Contrary to the statement on page 7, exemplification has been given in a prophetic example where the polymerase (and toxic gene) is the T7 RNA polymerase, an SV40 intron is chosen for the disrupting sequence, a compatible cell would be a mammalian cell and an incompatible cell would be E.coli. Methods of cloning the polymerase, the source of the intron sequences and sites in the gene and a potential eukaryotic vector for expression are all given in Example 19. Appropriate sequences are given in Figure 24 and the resultant product is shown in Figure 25. Further examples of construction steps are given in Figures 26-33. This extensive exemplification should not be considered to be a "minimal prophetic description".

Applicants further take issue with respect to assertions made regarding Balvay. Specifically, the Balvay et al. reference cited in the Office Action is better suited for a post-hoc explanation of why a particular site did not work rather than a predictive tool of why something is unlikely to work. Balvay et al. has no description of predictability or unpredictability of where a splice site will take place and only concerns itself with discussions of natural splice sites in natural genes.

Claims 246-254 depend from claim 245; claim 258 depends from claim 255 and claims 266-267 depend from claim 262. Therefore arguments made with respect to claims 245, 255 and 262 would apply to the dependent claims as well.

In view of the above amendments and arguments, Applicants assert that the rejections under 35 USC 112, written description have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

2. The Rejections Under 35 USC 112, First Paragraph (Enablement)

Claims 245-255, 258 and 262-267 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Office Action specifically states:

Applicants' specifically claim that the inserted and inactivating intronic sequences will be spliced out, a process the specification indicates will be carried out by the cellular machinery that normally operates to splice introns out of pre-mRNA sequences. Applicants indicate that such splicing restores native activity to previously inactive proteins. However, the specification as filed does not provide any nucleic acid constructs for which this has actually been shown. Applicants specification does not provide sufficient guidance or examples that would enable a skilled artisan to make the disclosed nucleic acid constructs containing sequences that are spliced out by cellular machinery. Although the specification prophetically considers and discloses making and using such constructs, such a disclosure would not be considered enabling since introducing intervening sequences into nucleic acids alters their secondary structure, which makes their ability to be cleaved by the splicing machinery unpredictable. The specification has not resolved such issues, since no exemplified constructs that contain intervening sequences and are inactive therefore, and by which later processing inside the cell restores activity. Applicants have simply not shown that such intervening sequences can be spliced out to restore any activity to previously inactive polymerases (or any toxic protein for that matter).

Although applicant asserts that the Balvay et al. reference is not applicable because it merely provides evidence that secondary structures may be involved and describes exceptional situations that may be encountered, Balvay et al. is considered to support the unpredictability of secondary structures of RNA and that such secondary structures have a pronounced effect on RNA splicing. Whether applicant considers the teachings of Balvay et al. to be regarding exceptional circumstances or not, Balvay *et* al. teach the complexity of secondary structures with regards to splicing and there is nothing in the instant specification or claims that excludes that teachings of Balvay et al. from being applicable.

In particular, it is demonstrated that the complex secondary structures of nucleic acids are responsible for their intron excision activity, and furthermore, that predicting the ability of the cellular splicing machinery to splice out precise intervening sequences from disrupted sequences with variable secondary structures such that native activity is restored is considered unpredictable, because the splicing

machinery is sensitive to the presence or absence of such structures.

Applicant relies on Lewin for teachings regarding experiments of splicing out a hybrid intron and teachings that splicing sites are generic, meaning that they do not have specificity for individual RNA precursors and the RNA precursors do not convey specific information (such as secondary structure) that is needed for splicing. The teachings of Lewin et al. do not diminish the unpredictability of the intron splicing mechanism when a non-native intron is inserted into a sequence having secondary structure. Simply because splice sites are generic to different sequences that do not "convey secondary structure that is needed for splicing does not mean that the mechanism does not encounter problems of unpredictability as taught by Balvay et al.

Furthermore, one of ordinary skill in the art would not be able to recognize which cells are "incompatible" or "compatible", as instantly recited, in view of the teachings of Lewin et al. that are cited by applicant. Specifically, if splicing sites are generic and do not have specificity for individual RNA precursors, as taught by Lewin et al., one would not be able to determine without undue experimentation how such introns would get excised from some cells and not from others, as instantly recited. The instant nucleic acid construct has to be able to allow excision of the intron in some cells but not in others.

Furthermore, the replacement of even a few nucleotides on an mRNA can abolish all activity of the translated protein. It is maintained that neither the specification nor the prior art arms one of skill with the information necessary to engineer sequences into nucleic acid constructs that will be reliably spliced out to result in a protein with native activity restored.

In order to practice the invention using the specification and the state of *the* prior art as outlined above, the quantity of experimentation required to practice the invention as claimed would therefore require the *de novo* determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might interfere with native activity. In the absence of any real guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.

Applicants respectfully traverse the rejection. *Contra* to assertions made in the Office Action, the instant specification combined with knowledge of one skilled in the art as of the priority date of the instant invention would provide sufficient guidance that would enable a skilled artisan to make the disclosed nucleic acid constructs contain sequences that are spliced out by cellular machinery. In particular, as noted above, Example 19 provides a more than adequate teaching of a construct containing a polymerase (and toxic gene) and eukaryotic (non-native) intron and source of these sequences. This example provides a more than sufficient description regarding strategies used in choosing intron sequences to be used, insertion sites in the T7 polymerase gene and vectors. Construction steps are exemplified in Figures 26-33. This in Applicants view would provide a sufficient roadmap to one of skilled in the art for obtaining the claimed constructs.

Further, as argued in the previous response filed, methods for well known in the art for introducing "artificial introns' into new environments. Two examples are referenced in the specification, Schwartz et al. 1993 (page 4) Mayeda et al. 1990 (page 85). One other example of an "artificial intron" includes Gatermann et al., 1989, Mol. Cell Biol. 9:1526-1535 (Exhibit 2) which discloses inserting an artificial intron into multiple sites. Applicants note that in contrast to the construct of the present invention, the small cassette in Gatermann included the minimal sequences that would constitute the post-splice junctions. However, in Gatermann, these sequences were inserted into several different locations without a problem. It was actually stated in the Gatermann abstract "the proper signals within an intron are sufficient to initiate and complete a splicing event independent of the location of the intron in the gene". Applicants further note that the yeast used in Gatermann (*S. pombe*) was observed to splice out the intron of SV40 correctly. A second example, Yoshimatsu and Nagawa, 1989 Science 244; 1346-1348 (Exhibit 3) used an intron cassette to control gene expression. The Yoshimatsu abstract states: "any gene can be converted to a controllable gene by simple insertion of an intron".

Applicants also take issue with assertions made that Balvay supports the unpredictability of secondary structures of RNA. Applicants note that Balvay in the last page of the article states "It is important to stress that in the absence of in vivo

experiments or in vitro systems where transcription and splicing are coupled, all these conclusions about the functional significance of secondary structure should be taken as tentative ones." Clearly, this is a tentative conclusion as opposed to practical exercises that have been carried out generating in vivo data that introduction of introns into selected sites is a predictable art with a high likelihood of success.

In contrast to the position stated in the Office Action, there is ample evidence that insertion of a selected intron cassette into a particular site should generate a predictable spliced product as exemplified by the four papers cited previously. In contrast there is no evidence in Balvay et al. that directly contradicts this and the authors themselves acknowledge a lack of working examples that tested their concepts. Applicants disagree with the position taken on page 10 that is prefaced with "Whether the applicants consider the teachings of Balvay et al. to be regarding exceptional circumstances or not....." In Applicants view, it is important whether Balvay was exceptional since it goes directly to the heart of predictability, i.e. the existence of exceptional cases does not affect the predictability of a method. It is well established case law that 100% certainty is not required and that it is sufficient that it be predictable enough to be able to avoid an undue amount of experimentation. Balvay's description of certain splicing cases was based upon the understanding that they were selected because they were exceptions that were in dire need of explanations as to why they did not follow the predicted rules. Balvay offers no teachings on the likelihood or unlikelihood of success of the present invention.

Applicants with respect to assertions made on page 11, assert that they were not simply relying on a textbook, Lewin, for theoretically proposing that there would be no problem. Applicants in the instant response and in the response to the previous Office Action cited research papers where introns were introduced into new environments without any problems being encountered.

Finally, Applicants take issue with the assertion that one of skill in the art would not be able to recognize compatible and incompatible cells. Splicing would not be able to occur in incompatible cells but could occur in compatible cells. However, in order to advance prosecution, claims 245, 255 and 262 have been amended to recite that the

polymerase/gene product is not expressed in the prokaryotic cell and is expressed in a eukaryotic cell.

Applicants note that claims 246-254, 258, and 265 are dependent claims. Thus arguments made with respect to the independent claims 245, 255 and 262 would apply to these claims as well. Furthermore, claims 263-264 and 266-267 have been canceled.

In view of the above amendments and arguments, Applicants assert that the rejections under 35 USC 112, first paragraph have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

4. Claim Rejections 35 USC 112, First Paragraph (New Matter)

Claims 263, 266 and 267 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The Office Action specifically states

> Claims 263, 266 and 267 were newly added with the claims filed on 1/3/07. Claims *263* and 267 recite, "wherein said incompatible cell is a prokaryotic cell and said compatible cell is a eukaryotic cell" and claim 266 recites "wherein said incompatible cell is a prokaryotic cell". These limitations are considered new matter because the specification does not specifically disclose that the incompatible cells are prokaryotic cells and the compatible cells are eukaryotic cells.

Applicants respectfully traverse the rejection. Before responding, claims 263, 266 and 267 have been canceled. However, claims 245, 255 and 262 have been amended to recite that the gene product is incapable of being expressed in a prokaryotic cell and that the intron is removed during processing in a eukaryotic cell. Applicants assert that these claim amendments are supported by the specification. Lack of removal of the intron used in the construct of the present invention is described on page 85:

> It is possible that insertion of a heterologous processing element may not in all cases inactivate a gene when present in an incompatible cell. Although splicing has been observed in prokaryotic systems for bacteriophage T4 and thus independent of processes employed in compatible cells.

> Therefore, in a prokaryotic environment, the intron should remain in the mRNA as long as a self-splicing intron is not used.

An example of the construct recited in claim 245 is described in Example 19. A description of this example is provided on page 89. It is stated:

> The present invention (see Examples) describes the conditional inactivation of a gene (that normally does not contain a processing element) by the precise introduction of an intron between the last two G's of a site that has the post splice junction sequence (C/A)AGG. The introduction of an intron into sites with this sequence creates a functional splice donor and a functional spice acceptor. Therefore, a construct with this modification could lack any expression of T7 RNA polymerase in an E. coli cell, but the normal coding sequence can be restored from transcripts after introduction into a compatible cell.

In Example 19, particularly page 152, it is stated "This particular eukaryotic vector was chosen since it had been shown previously that the RSV promoter is especially active in hematopoietic cell lines". Hematopoietic cell lines are eukaryotic cells. Furthermore, Figure 25 referenced in Example 19 states "Active T7 RNA polymerase is only made in eukaryotic cells after splicing out of SV40 intron".

In view of the above arguments and amendments of the claims, Applicants assert that the rejections of claims 263, 266 and 267 have been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

Summary and Conclusions

It is Applicants belief that the pending claims are in condition for allowance. However, if a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

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